Bernadeta Pietrzak-Wawrzyńska, MSc

**Extranuclear-Initiated Estrogen Receptor Signaling as a Novel Target for Neuroprotective Therapies: Identification of the Molecular Mechanisms of PaPE-1 Action in Primary Neuronal Cultures and HMC3 Microglial Cells Exposed to Aβ**

ABSTRACT

Alzheimer's disease is a neurodegenerative disorder affecting millions of people worldwide, constituting a significant health, social, and economic burden. The disease affects women more frequently, especially postmenopausal women, suggesting a significant role for sex hormones, particularly estrogens, in its pathogenesis. The protective effects of estrogens make them an attractive option for the prevention and treatment of Alzheimer's disease, but their clinical use carries the risk of serious side effects, such as hormone-dependent cancers and cardiovascular disease. These effects of estrogens are associated with nonselective activation of classical nuclear estrogen receptor signaling pathways and the induction of hormonal effects outside the central nervous system. These limitations highlight the need for new ligands with a more selective mode of action, capable of harnessing the neuroprotective effects of estrogens while minimizing severe side effects. PaPE-1 (Pathway Preferential Estrogen), a novel ligand for the ESR1 and ESR2 receptors that selectively activates extranuclear estrogen signaling, may represent a promising approach to achieve this goal.

Amyloid-β (Aβ) is one of the key factors involved in the pathogenesis of Alzheimer's disease. Our previous studies have shown that PaPE-1 administered concomitantly with Aβ, i.e., in the co-treatment paradigm, has a protective effect, mitigating Aβ-induced neurotoxicity, apoptosis, and oxidative stress. In this doctoral dissertation, I decided to investigate whether PaPE-1 exerts neuroprotective effects in a delayed, post-treatment paradigm. This timeframe better reflects the clinical setting in which the drug is administered when neuropathology is already present. To verify the protective potential of PaPE-1 and understand its mechanisms of action, I used primary cultures of mouse neuronal cells and the human microglial cell line HMC3. These cells were first exposed to aggregated Aβ (5 or 10 μM) for 24 hours and then treated with PaPE-1 for 6 and/or 24 hours. I demonstrated that PaPE-1 (10 μM) administered with a 24-hour delay from Aβ effectively protects neuronal cells and modulates the inflammatory response of microglia. In neuronal cells, PaPE-1 partially reverses Aβ-induced changes, reducing neurodegeneration and increasing neurite network density. This is accompanied by a decrease in the expression of factors important in the pathogenesis of Alzheimer's disease, such as *App*, *Apoe*, *Bace2*, and *Rbfox1*, as well as upregulation of *Chat* expression. The protective effects of PaPE-1 are associated with limited apoptosis, which is manifested by a decrease in caspase-3 and -9 activity, a reduction of apoptotic chromatin condensation, as well as by downregulation of proapoptotic factors (*Bax*/BAX,

*Gsk3b*, *Fas*/FAS, and *Fasl*/FASL). Furthermore, the tested ligand normalizes autophagy weakened by Aβ, as evidenced by an increase in the level of autophagic vesicles and an upregulation of key factors in this process (*Becn1*/BECN1, *Atg5*, *Atg7*, *Ambra1*, and *Map1lc3b*/Map1LC3AB). PaPE-1 also increases the expression of ESR1 and ESR2 receptors in the membrane fraction, and the use of selective estrogen receptor antagonists indicates that the ESR1 is required for its effects. PaPE-1 exhibits epigenetic modulator properties, stimulating the activity of histone acetylases (HAT) and modifying DNA methylation levels in the promoter regions of numerous genes, including those involved in apoptosis, autophagy, and estrogen signaling. Furthermore, PaPE-1 is capable of modulating neuroinflammation, as demonstrated in neuronal and microglial cells exposed to Aβ. In neuronal cells, the tested compound effectively reduces biochemical and molecular markers of the inflammatory response, such as caspase-1 activity, extracellular ATP concentration, and expression of inflammatory factors. In microglia, PaPE-1 partially normalizes the changes occurring in response to Aβ, including cell morphology, metabolic activity, and IL1β level.

My research provides the first evidence that post-treatment with PaPE-1 exerts neuroprotective effects and modulates neuroinflammatory responses in cellular models of Alzheimer's disease. Based on the obtained results, I suggest that therapeutic approach based on the selective activation of extranuclear estrogen signaling may become an effective and safe strategy in the future fight against Alzheimer's disease.